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Effects of bovine and caprine Monterey Jack cheeses fortified with milk calcium on bone mineralization in rats

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Abstract

Bovine and caprine Monterey Jack cheeses were produced unfortified (cheese) or fortified with milk calcium (Ca-cheese). Five groups of male rats were fed a control diet or one of four experimental diets: bovine cheese diet, bovine Ca-cheese diet, caprine cheese diet, and caprine Ca-cheese diet to test their effects on calcium absorption as well as bone mineralization. Significant differences ($P<0.05$) were found for calcium absorption and digestibility with the data yielding the pattern: caprine Ca-cheese > caprine cheese = bovine Ca-cheese > bovine cheese > control. Significant increases ($P<0.05$) occurred in bone mineral content (BMC), bone mineral density (BMD) and breaking force in the femoral bone with the pattern: caprine Ca-cheese > caprine cheese > bovine Ca-cheese > bovine cheese > control. Our findings suggest that caprine cheese unfortified or fortified with milk calcium had the most positive effects on calcium absorption and bone mineralization in rats, with bovine cheeses still having significantly greater effects than the non-casein control.

Keywords: Bovine cheese; Caprine cheese; Milk calcium; Ca bioavailability

1. Introduction

Dietary calcium deficiency has been linked epidemiologically to several chronic diseases including osteoporosis, osteomalacia, hypertension, colon cancer, and obesity (Berner, McBean, & Lofgren, 1990; Tunick, 1987; Zemel & Miller, 2004). Osteoporosis is defined as bone loss sufficient to bring about life-threatening fractures, especially for those who are elderly. Women, in general, are more prone to osteoporosis than are men because of their smaller skeletal mass at maturity and because of the period of rapid bone loss that often occurs following the menopause. Insufficient calcium intake is a major contributing factor (Weaver, 1992). However, magnesium, in concert with calcium and vitamin D, aid in the complicated process that builds up strong bones (Okuma, 2001).

When geographic or population-based calcium deficiencies have been recognized, restoration of calcium to the food supply of the affected groups has soon followed (NIH, 1994). Most studies have found the calcium availability from various salts to be virtually identical (Heaney, Weaver, Fitzsimmons, & Recker, 1990). However, milk calcium is more absorbable than any other inorganic calcium source and calcium in dairy products is more readily absorbable and bioavailable than calcium from meats, poultry, fish, eggs, grains, nuts, beans, and green leafy vegetables (Heaney, 2000). One reason for this may be that the major portion of calcium in milk (approximately 65%) and a part of calcium in cheese is complexed with the phosphoproteins in the colloidal casein micelles (Farrell, 1999). Colloidal calcium and calcium caseinates are more absorbable and bioavailable than ionic calcium, presumably due to the chemical form of calcium in milk (Igarashi & Ezawa, 1991; Kato, Toba, Takada, & Aoe, 1997). In vivo production of phosphopeptides from casein micelles by trypsin and other proteolytic enzymes in

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the gut may promote calcium uptake through intestinal cells by preventing precipitation of insoluble calcium (FitzGerald, 1998). Phosphopeptides have been linked to enhanced calcium absorption from calcium-fortified bovine milk (Tsuchita, Suzuki, & Kuwata, 2001).

The health benefits of additional amounts of calcium in the form of milk calcium have been investigated by Kato et al. (1997) and Kato, Takada et al. (2002). Their research suggest that milk calcium used as a food fortificant in bovine milk powder or bovine cheese contributes to an increase in bone density and bone mechanical strength in laboratory animals. If verified by other reports, this finding implies that the fortification of dairy products with milk calcium actually may aid in preventing the fracture of bones (osteoporosis). This increase in calcium uptake from fortified milk (and fortified cheese) could be the result, in part, of the generation of phosphopeptides from caseins.

In bovine milk, the constancy of the ratio of caseins (α_{s1}, α_{s2}, β, κ) results in a rather uniform distribution of structure of the casein micelles and yields cheeses with consistent technological properties (rennet gel strength and gel firming rate). However, the variability of the casein ratios in caprine milks, α_{s1}- to α_{s2}-casein in particular, can affect micelle structure and possibly calcium availability. Accordingly this study will compare cheeses from normal bovine milk (α_{s1}- to α_{s2}-ratio of 3:1) to those from selected caprine milks (α_{s1}- to α_{s2}-ratio of 1:5; Mora-Gutierrez & Farrell, 2001). In addition, casein phosphopeptides (CPP) from bovine and caprine cheeses have not been characterized comparatively relative to their interaction with milk calcium.

Therefore, the objective of this study was to assess calcium absorption and deposition in male rats fed diets containing caprine Monterey Jack cheese unfortified or fortified with milk calcium. Diets containing bovine Monterey Jack cheese unfortified or fortified with milk calcium were also used for comparison purposes, along with a non-casein control diet. Differences arising among the cheese groups and control will be related to differences between the caprine and bovine milk compositions.

2. Materials and methods

2.1. Milk samples for cheesemaking

Bovine milk was obtained from individual Jersey cows of a privately owned dairy farm, Hempstead, TX, USA. Caprine milk was obtained from individual French-Alpine goats which were screened for high content of α_{s2}-casein at the International Dairy Goat Research Center, Prairie View A&M University, Prairie View, TX, USA. The cows and goats were in midlactation. The milk was analyzed (AOAC, 1995) to determine the protein content (bovine: 3.29%; caprine: 3.28%), fat content (bovine: 3.71%; caprine: 3.79%), and lactose content (bovine: 4.69%; caprine: 4.53%). Calcium content (bovine: 0.119%; caprine: 0.109%) and phosphorus content (bovine: 0.255%; caprine: 0.262%) of these milks were determined according to methods described by Cerbulis and Farrell (1976). The ratio of casein to whey protein in the bovine and caprine milk was 5.32 and 5.49, respectively, as determined by SDS-PAGE electrophoresis and densitometry (Basch, Douglas, Procino, Holsinger, & Farrell, 1985). The concentrations of vitamin D in the bovine and caprine milk samples were 24.1 and 24.7 IU, respectively (AOAC, 1995).

The various components of milk fat, fatty acids, differ in carbon chain length and saturation. Caprine milk fat normally has 35% of medium-chain fatty acids (C6–C14) compared to bovine milk fat 17%, and three are named after goats: caproic (C6), caprylic (C8), capric (C10), totaling 15% in caprine milk fat versus 5% in bovine milk fat. The concentrations of medium-chain fatty acids in the fat fraction of the bovine and caprine milks used to prepare the cheeses of the present study were 19.3% and 35.1%, respectively, as determined by gas chromatography (Schroeder et al., 2003).

2.2. Preparation of milk calcium

Milk calcium was prepared from the whey serum fraction of bovine milk according to the method of Kato, Toba et al. (2002). Briefly, fresh bovine milk was obtained as a pooled sample from a herd of Jersey cows. The bovine milk was skimmed by centrifugation at $4000 \times g$ for 15 min at 4 °C and adjusted to pH 4.2 with 1 M HCl. The casein fraction was separated by centrifugation at $3000 \times g$. The supernatant was concentrated 10-fold by evaporation, cooled to 4 °C for 12 h, and the lactose was precipitated and separated by centrifugation at $3000 \times g$. The supernatant was adjusted to pH 6.8 with 1 M NaOH and centrifuged at $3000 \times g$. The precipitate was collected and washed with deionized water and the whey protein was partially removed by ultrafiltration through a membrane with a molecular mass cut-off of 100 kDa using an Amicon hollow fiber concentrator (Amicon, 72 Cherry Hill Drive, Beverly, MA, USA). The retentate was collected and spray-dried. The proteins present in the preparation were whey proteins (mainly β-lactoglobulin and α-lactalbumin) (Farrell, 1999).

Milk calcium was analyzed (AOAC, 1995) to determine the moisture content (4.6%), the protein content (8.1%), the fat content (0.4%), the carbohydrate content (8.9%), and the ash content (78%). Calcium content (15.2%), magnesium content (0.6%), and phosphorus content (8.9%) of milk calcium were determined according to methods described by Cerbulis and Farrell (1976).

2.3. Composition of bovine and caprine cheeses unfortified or fortified with milk calcium

The bovine and caprine cheeses unfortified or fortified with milk calcium were made with milk samples of known casein composition: α_{s2}-casein (bovine milk:

12.1%; caprine milk: 29.2%), α_{s1}-casein (bovine milk: 39.5%; caprine milk: 5.9%); β-casein (bovine milk: 37.2%; caprine milk: 50.5%) and κ-casein (bovine milk: 11.2%; caprine milk: 14.4%). Milk calcium (0.5 g 100 g^{-1} Ca) was mixed by continuous stirring for 45 min at 4 °C. The milk was stored at 4 °C, homogenized (1st stage −20 M Pa and 2nd stage −10 MPa), pasteurized (63 °C; 30 min) and processed into Monterey Jack cheese according to methods described by Attaie (2005).

An approximately 100 g triangular slab of each cheese was cut from outside toward the center of each wheel and used for analyses. Moisture, which is a measure of yield and quantity of food solids, was determined in finely ground representative samples of 1-week-old cheese by oven-drying at 105 °C for 24 h (AOAC, 1995) and cross-checked with the vacuum drying method (65 °C for 24 h). The concentration of milk fat was assayed using the Babcock method (Richardson, 1985). Five-gram samples of each cheese were mixed with 20 mL of double-ionized water and homogenized at high speed (Biospec Product, Inc., Bartlesville, OK, USA) for 2 min. The cheese slurry was transferred into centrifuge tubes and incubated in water at 40 °C for 1 h. The homogenate was centrifuged at 3000 × g for 30 min. The supernatant was filtered through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, England) and 5 mL of the filtrate used for N analysis by the Kjeldahl procedure (AOAC, 1995). Carbohydrate content was determined by difference of fat, protein and ash from total solids (Richardson, 1985). For determination of mineral concentrations, 5 g of cheese samples were dry ashed in a porcelain crucible, solubilized with 10 mL of 6 N HCl, quantitatively transferred into 25-mL volumetric flasks, and diluted to volume with double ionized water. Concentrations of major minerals in the samples were determined by Inductively Coupled Argon Plasma Emission Spectroscopy (model number Atom-Comp-1100; Jarrell-Ash Co., Franklin, MA, USA). The sample flow rate was 0.625 L min^{-1}, and the nebulizer pressure was set at 40 psi. The wavelengths used for the tested minerals were: P, 214.9; K, 766.2; Na, 588.9 or 330.2; Mg, 279.0 or 279.5; Ca, 315.8 or 317.9 nm, respectively.

2.4. Electrophoresis

Samples of 2 g were cut from each cheese and individually vacuum-packaged. After each storage period (0, 14, 28, 42, and 56 days) at 4 °C, the individually packaged cheese samples were opened, finely ground, and thoroughly mixed before electrophoresis. The extend of proteolysis of the casein peptides in the cheeses was measured by polyacrylamide gel electrophoresis (PAGE) in sodium dodecyl sulfate (SDS), and densitometry was used to assess the relative concentrations of casein peptides (Basch, Farrell, Walsh, Konstance, & Kumosinski, 1989). Casein peptide bands were identified by comparing with those of bovine and caprine milk casein standards: α_{s1}-, α_{s2}-, β-, and κ-casein.

2.5. Diets

The composition of the pre-experimental diet was as follows: 20.0% casein, 15.0% corn starch, 5.0% cellulose, 5.0% corn oil, 50.2% sucrose, 0.3% DL-methionine, 1.0% AIN-76 vitamin mixture and 3.5% AIN-76 mineral mixture. The pre-experimental diet was prepared according to the recommendations of the AIN-76 (American Institute of Nutrition, 1977).

During the experimental period, each group of animals (control group, bovine cheese group, bovine Ca-cheese group, caprine cheese group, and caprine Ca-cheese group) was given two types of experimental diets (Types I and II diets). The experimental diets (Types I and II) given to the five groups are described in Table 1. In the control diet (control group), soy protein was used as the sole source of protein, and calcium carbonate and milk calcium were used as the sources of calcium (Type I diet: calcium carbonate 0.5%, milk calcium 1.1%; Type II diet: calcium carbonate 0.5%). In the bovine or caprine cheese diet (bovine or caprine cheese group), 20% soy protein isolate and 0.50% calcium carbonate in the control diet were replaced by unfortified bovine or caprine cheese because the same amount of milk calcium was fed separately (Type I diet: calcium carbonate 0.5%, milk calcium 1.1%; Type II diet: bovine or caprine cheese 35%). In the bovine or caprine Ca-cheese diet (bovine or caprine Ca-cheese group), 20% soy protein isolate, 0.50% calcium carbonate, and 1.1% milk calcium in the control diet were replaced by bovine or caprine cheese fortified with milk calcium because milk calcium and bovine or caprine cheese were fed at the same time (Type I diet: bovine or caprine cheese fortified with milk calcium 35%; Type II diet: calcium carbonate 0.5%). The amounts of protein, sulfur-containing amino acids, calcium, phosphorus, and magnesium were quite similar in the five experimental diets (control, bovine cheese, bovine Ca-cheese, caprine cheese, and caprine Ca-cheese) consisting of Types I and II diets each. The bovine and caprine cheeses used to prepare the cheese diets displayed a ripening period of 14 days at the beginning of the animal feeding study. All experimental diets were stored at 4 °C after processing and during the 28-days feeding experiment.

2.6. Animal feeding experiments

Three-week old male Sprague–Dawley rats (Harlan Teklad, Madison, WI, USA) were housed individually in stainless-steel mesh bottom cages in a temperature-controlled (23 ± 2 °C) room with 45% ± 10% humidity and a 12-h light–dark cycle. The animals were fed the AIN-76 diet (American Institute of Nutrition, 1977). In the pre-experimental period, rats for the meal-feeding experiment were trained to consume an AIN-76 diet within 2 h (twice per day) and given free access to double deionized water (Milli-Q Biocel, Ultrapure Water System, Millipore Corporation, Billerica, MA, USA) for 6 weeks.

Table 1
Composition of experimental diets (%)

	Control		Bovine cheese		Bovine Ca-cheese		Caprine cheese		Caprine Ca-cheese	
	Type I	Type II	Type I	Type II	Type I	Type II	Type I	Type II	Type I	Type II
Soy protein isolate[a]	20.01	20.01	20.01	12.17	12.24	20.01	20.01	12.21	12.24	20.01
Cheese	—	—	—	35.00	—	—	—	35.00	—	—
Cheese fortified with milk calcium	—	—	—	—	35.00	—	—	—	35.00	—
Cornstarch	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Cellulose	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Corn oil	9.45	9.49	9.45	—	—	9.49	9.45	—	—	9.49
Sucrose	45.40	45.74	45.40	30.20	30.39	45.74	45.40	30.17	30.40	45.74
L-Cysteine	0.10	0.10	0.10	0.05	0.05	0.10	0.10	0.05	0.05	0.10
DL-Methionine	0.65	0.65	0.65	0.55	0.55	0.65	0.65	0.55	0.55	0.65
Milk calcium	1.12	—	1.12	—	—	—	1.12	—	—	—
$CaCO_3$	0.52	0.54	0.52	—	—	0.54	0.52	—	—	0.54
NaCl	0.39	0.45	0.39	—	—	0.45	0.39	—	—	0.45
Potassium citrate	0.36	—	0.36	0.63	0.20	—	0.36	0.63	0.20	—
Citric acid monohydrate	0.06	0.50	0.06	0.09	—	0.50	0.06	0.09	—	0.50
KH_2PO_4	0.61	1.09	0.61	—	—	1.09	0.61	—	—	1.09
K_2CO_3	—	—	—	—	0.27	—	—	—	0.27	—
Na_2CO_3	0.12	0.21	0.12	—	—	0.21	0.12	—	—	0.21
K_2SO_4	—	—	—	0.18	0.18	—	—	0.18	0.18	—
$MgSO_4$	0.13	0.13	0.13	—	—	0.13	0.13	—	—	0.13
MgO	0.01	0.02	0.01	0.06	0.05	0.02	0.01	0.05	0.04	0.02
Vitamin mixture[b]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Basal mineral mixture[c]	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07

[a]Dupont Protein Technologies, St. Louis, MO.
[b]Identical to AIN-76 vitamin mixture.
[c]Contained 17.5% $MnCO_3$, 30.0% Fe-citrate (Fe 17%), 8.0% $ZnCO_3$, 1.5% $CuCO_3$, 0.05% $Na_2SO_3 \cdot 5H_2O$, 0.05% KIO_3, and $CrK(SO_4)_2 \cdot 12H_2O$.

After the 6-week period, the animals were allotted by body weight to five groups of 7 rats each so that the mean body weight of rats in each group ranged from 201 to 203 g. All rats were alternately given the Type I diet (morning on the odd-numbered days and evening on the even-numbered days) and the Type II diet (evening on the odd-numbered days and morning on the even-numbered days) for each of the 28 days of a 2-h meal-feeding (twice per day). They were also given free access to double deionized water for 28 days. Body weight was recorded once a week, and food intake was monitored daily. Feces were collected on the last 2 days of the feeding period and kept frozen (-21 ± 1 °C) until analyzed for calcium and magnesium. At the end of the 28-day trial period, the rats were deprived of food overnight and anesthetized by intraperitoneal injection of sodium pentobarbital (40 mg kg^{-1} body weight). Blood was withdrawn by cardiac puncture and collected in tubes with no additives. The tubes were centrifuged at $1400 \times g$ for 15 min to obtain the serum, which was kept frozen (-21 ± 1 °C) until analyzed for calcium and magnesium within a 1-week period. After the rats were killed, both femurs were excised from each rat and the surrounding flesh removed. Femurs were stored at -83 °C until analyzed.

All management and experimental procedures were carried out in strict accordance with the current guidelines and legal requirements established in the United States for the proper care and use of laboratory animals.

2.7. Analytical methods

Diets, feces, and femoral bones were dry-ashed in a muffle furnace (Barnstead/Thermolyne Corp., Dubuque, IA, USA) at 550 °C. Ashes were dissolved in an HCl/HNO_3/H_2O solution (1 + 1 + 2) (Sigma Chemical Co., St. Louis, MO, USA). Calcium and magnesium analyses of diets, feces, and femoral bones were performed by means of flame atomic absorption spectrometry with a Varian SpectrAA 55 (Walnut Creek, CA, USA) using 0.5% lanthanum ($LaCl_3$, Sigma Chemical Co.) to avoid interferences. Calcium and magnesium atomic standard solutions (995 μg Ca mL^{-1} in 1% HCl or 995 μg Mg mL^{-1} in 1% HCl) (Sigma Chemical Co.) were used. A blank solution with lanthanum was also used.

Given the importance of accurate determination of calcium and magnesium in diets, feces and femoral bones of this study, the measurement of these was subjected to a quality control procedure. This consisted of analysing a skimmed bovine milk powder (certified reference material CRM 63; Community Bureau of References, Brussels, Belgium), which yielded a calcium value of 12.8 ± 0.2 mg g^{-1} (mean ± SD of five determinations) (certified value of calcium, 12.6 ± 0.3 mg g^{-1}) and a magnesium value of 1.21 ± 0.07 mg g^{-1} (mean ± SD of five determinations; certified value of magnesium, 1.26 ± 0.02 mg g^{-1}).

Serum calcium was measured by a colorimetric procedure (Michaylova & Ilkova, 1971). Serum magnesium

was measured by a kinetic colorimetric procedure (Wimmer, Artiss, & Zak, 1986). Bone phosphorus was determined by a micro phosphate method (Dieter, Meun, & Smith, 1968).

2.8. Indices

Food efficiency

= Body mass gain/Food intake in dry matter.

The following indices were calculated from data on calcium and magnesium intake and fecal calcium and magnesium excretion:

Apparent absorption = Intake − Fecal excretion

$\%A/I$ = Apparent absorption/Intake × 100.

2.9. Bone measurements

The femoral bones were scanned on a bone densitometer (Lunar Corp., Madison, WI, USA) using the small animal software (Luna DPX-IQ model, Lunar Corp.) to determine the bone mineral content (BMC) and bone mineral density (BMD). The mechanical property of the femoral bone was determined by a three-point bending test on a Texture Analyzer (model QTS-25, CNS Farnell, Ltd., Hertfordshire, England) as described by Raab, Smith, Crenshaw, and Thomas (1990).

2.10. Statistical analysis

Statistical analyses of the distribution of casein peptides present in the bovine and caprine cheeses during the ripening period were carried out using the PROC general linear model procedure of Statistical Analysis Systems (SAS, 1999; SAS Institute, Inc., Cary, NC, USA):

$$Y = \mu + C_j + T_i + (C * T)_{ij} + \varepsilon_{ij},$$

where Y is the mean value of each variable tested, μ the population mean, C_j the cheese type ($j = 1-4$; 1 = bovine cheese, 2 = bovine cheese + calcium, 3 = caprine cheese, and 4 = caprine cheese + calcium), T_i the time ($i = 1-5$; 1 = 0 day, 2 = 14 days, 3 = 28 days, 4 = 42 days, and 5 = 56 days), $C*T$ the interaction of time and treatments, and ε the error term, the random variable assumed to be normally distributed with mean equal to zero and constant variance.

Duncan multiple range test was used to compare means that showed significant variation ($P<0.05$).

The results of mineral concentrations in diets, feces, femoral bones and the biomechanical properties of femoral bones were tested statistically by one-way analysis of variance (ANOVA), followed by Duncan's test to compare means that showed significant variation ($P<0.05$):

$$Y = \mu + T_k + \varepsilon_k,$$

where Y is the mean value of each variable tested, μ the population mean, T_k the treatments ($k = 1-5$; 1 = control, 2 = bovine cheese, 3 = bovine cheese + calcium, 4 = caprine cheese, and 5 = caprine cheese + calcium), and ε the error term, assumed to be normally distributed with mean equal to zero and constant variance.

3. Results

3.1. Proteolysis of cheeses

Degradation patterns of casein peptide moieties by electrophoretic analysis (Table 2) revealed that similarities existed in proteolytic patterns of the casein peptides in the bovine and caprine Monterey Jack cheeses stored at 4 °C. The PAGE characteristics of casein peptide moieties of caprine and bovine Monterey Jack cheeses showed that the relative intensities of peptide bands mirrored those of the parent milks with respect to the gross casein contents, as expected for the caprine cheeses, the α_{s2}- and β-casein peptide bands were much greater than those of α_{s1}-casein peptides. For both bovine and caprine cheeses these bands gradually became weaker as the aging progressed; between zero and 14 days the intensity of all bands decreased, but remained quite stable between 14 and 28 days. Then the intensity of the α_{s2}-, α_{s1}-, and β-casein peptide bands in the bovine and caprine Monterey Jack cheeses significantly decreased after the 42 days of ripening ($P<0.05$). This is important because the experimental diets were compounded initially at 14 days of ripening and the study was conducted for an additional 28 days, so the peptide patterns of the cheeses were stable during the experimental feeding period. It is also important to note that the intensities of α_{s2}-, α_{s1}-, and β-casein peptide bands when compared between bovine and caprine Monterey Jack cheeses remained significantly different ($P<0.05$) throughout the aging period.

The differences in the casein peptide profiles of the bovine and caprine Monterey Jack cheeses (Table 2) could affect the nutritional value of these cheeses as a source of calcium in terms of calcium absorption and utilization for bone mineralization. Therefore, we tested the hypothesis that differences in casein peptide variability (mainly α_{s1}-, α_{s2}-, and β-casein peptides) in bovine and caprine cheeses could alter the calcium bioavailability from milk calcium added to the bovine and caprine cheeses (Table 3).

The rats, which were divided into five experimental groups (control group, bovine cheese group, bovine Ca-cheese group, caprine cheese group, and caprine Ca-cheese group), were fed two types of experimental diets (Types I and II) at 2-h meal-feeding (twice per day) for 28 days (Table 1). The concurrent administration of milk calcium with the bovine and caprine cheese diets (fortified cheese) (Type II diet) was compared with those of the bovine and caprine cheese diets (unfortified cheese) and the control diet (Type I diet).

Table 2
Densitometric analysis of SDS-PAGE bands of caseins from bovine and caprine cheeses (μg g protein^{-1})[a]

Casein type cheese	Time (days)					
	0	14	28	42	56	SEM
α_{s2}-casein						
Bovine cheese	2.80^{ae}	2.34^{be}	2.28^{bce}	2.23^{ce}	2.08^{de}	0.029
Bovine Ca-cheese	2.76^{ae}	2.29^{be}	2.23^{bce}	2.18^{ce}	2.02^{de}	0.029
Caprine cheese	5.86^{af}	5.27^{bf}	5.18^{cf}	5.07^{df}	4.79^{df}	0.029
Caprine Ca-cheese	5.82^{af}	5.31^{bf}	5.24^{bf}	5.13^{cf}	4.72^{df}	0.029
α_{s1}-casein						
Bovine cheese	4.65^{ae}	3.98^{be}	3.90^{bce}	3.81^{ce}	3.48^{de}	0.036
Bovine Ca-cheese	4.68^{ae}	3.91^{be}	3.87^{bce}	3.76^{ce}	3.40^{de}	0.036
Caprine cheese	1.39^{af}	1.10^{bf}	1.08^{bf}	1.05^{bf}	0.92^{cf}	0.036
Caprine Ca-cheese	1.42^{af}	1.08^{bf}	1.05^{bf}	1.03^{bf}	0.88^{cf}	0.036
β-casein						
Bovine cheese	21.02^{ae}	18.16^{be}	17.98^{bce}	17.65^{ce}	16.436^{de}	0.016
Bovine Ca-cheese	20.98^{ae}	18.21^{be}	18.11^{be}	17.82^{be}	16.58^{ce}	0.016
Caprine cheese	30.96^{af}	27.31^{bf}	27.20^{bf}	26.93^{bf}	25.09^{cf}	0.016
Caprine Ca-cheese	30.92^{af}	27.25^{bf}	27.17^{bcf}	26.78^{cf}	25.12^{df}	0.016
κ-casein						
Bovine cheese	2.19^{ae}	2.03^{be}	1.96^{bce}	1.94^{ce}	1.82^{de}	0.031
Bovine Ca-cheese	2.13^{aef}	1.98^{beg}	1.91^{bef}	1.89^{be}	1.79 ^{ce}	0.031
Caprine cheese	2.08^{afg}	1.86^{bf}	1.83^{bcf}	1.75^{cf}	1.63^{df}	0.031
Caprine Ca-cheese	2.02^{ag}	1.90^{bfg}	1.86^{bcf}	1.79^{cf}	1.65^{df}	0.031

Means with no common superscripts (a,b,c,d) within a row differ ($P<0.05$). Means with no common superscripts (e,f,g) within a column differ ($P<0.05$).
[a]Mean values (±SEM; $n = 3$) are given.

Table 3
Compositions of bovine and caprine cheeses unfortified or fortified with milk calcium[a]

	Bovine cheese	Bovine Ca-cheese	Caprine cheese	Caprine Ca-cheese
Moisture (g 100 g^{-1})	41.6±0.8	41.9±0.5	41.5±0.6	41.6±0.7
Fat	27.1±0.3	27.0±0.5	27.1±0.2	27.1±0.4
Protein	22.4±0.5	22.2±0.2	22.3±0.3	22.2±0.1
Ash	4.7±0.2	5.1±0.3	4.5±0.1	4.8±0.1
Carbohydrate	4.2±0.4	3.8±0.2	4.6±0.5	4.3±0.3
Ca (mg 100 g^{-1})	661±0.01	1179±0.03	599±0.01	1121±0.04
P	977±0.01	1015±0.01	820±0.02	874±0.01
Mg	29±0.001	41±0.001	40±0.001	52±0.001
Na	1085±0.2	894±0.01	881±0.02	856±0.01
K	65±0.001	72±0.001	76±0.001	85±0.001

[a]Mean values (±SD; $n = 3$).

3.2. Biological assays

3.2.1. Body weight gain, food intake and food efficiency

The body weight gain, food intake, and food efficiency were not significantly different among the five experimental groups throughout the study (Table 4). The amounts of daily calcium and magnesium intake were quite similar among the five experimental groups of rats (Table 5). The amounts of daily phosphorus intake were quite similar among the following groups: control group, caprine cheese group, or caprine Ca-cheese group (Table 5). The bovine cheese and bovine Ca-cheese groups (Table 5) had phosphorus intakes significantly higher ($P<0.05$) than the control, caprine cheese, and caprine Ca-cheese groups because of the slightly higher phosphorous content of the cheeses (Table 3). Although the rats fed the bovine cheese and bovine Ca-cheese diets had a high intake of phosphorus, we chose to include them as a significant covariate for phosphorus.

3.2.2. Apparent calcium and magnesium absorption and digestibility

The group of rats fed the caprine Ca-cheese diet had the highest apparent Ca absorption ($P<0.05$) followed by the

Table 4
Body weight gain, food intake, and food efficiency ratio of rats fed the different diets for 28 days[a,b]

	Dietary group					
	Control	Bovine cheese	Bovine Ca-cheese	Caprine cheese	Caprine Ca-cheese	SEM
Body weight gain ($g\,day^{-1}$)	4.1	4.3	4.1	4.2	4.4	0.2
Food intake ($g\,day^{-1}$)	18.9	19.1	18.4	19.5	18.9	0.2
Food efficiency (%)	21.7	22.5	22.3	21.5	23.2	0.2

[a]See Table 1 and text for details of diet treatment.
[b]Mean values ($\pm$SEM; $n = 7$).

Table 5
Calcium and magnesium absorption and digestibility of rats fed the different diets for 28 days[a,b]

	Dietary group				
	Control	Bovine cheese	Bovine Ca-cheese	Caprine cheese	Caprine Ca-cheese
Ingested calcium ($mg\,d^{-1}$)	63.72d	64.37d	63.76d	63.61d	63.59d
Fecal calcium ($mg\,d^{-1}$)	37.48c	30.40d	27.23e	24.96e	20.28f
Absorbed calcium ($mg\,d^{-1}$)	26.24f	33.97e	36.53de	38.65d	43.31c
Calcium digestibility (% A/I)	41.18f	52.77e	57.29d	60.76d	68.11c
Ingested magnesium ($mg\,d^{-1}$)	9.20c	9.68c	9.12c	9.68c	9.14c
Fecal magnesium ($mg\,d^{-1}$)	5.94c	4.93d	4.75d	3.23e	2.92e
Absorbed magnesium ($mg\,d^{-1}$)	3.26e	4.75d	4.37d	6.45c	6.22c
Magnesium digestibility (% A/I)	35.43e	49.07d	49.92d	66.63c	68.05c
Phosphorus intake ($mg\,d^{-1}$)	76.24e	80.02d	79.25d	76.37e	76.73e

[a]See Table 1 and text for details of diet treatment.
[b]Mean values ($\pm$SD; $n = 7$). Means not sharing common letters within each row are significantly different ($P < 0.05$).

caprine cheese diet, which was statistically equal to the bovine Ca-cheese diet, and finally the bovine cheese diet. Note that the average calcium absorption for all cheese diets ($38.1\,mg\,d^{-1}$) was 1.5 times that of the control diet (Table 5) indicating that all cheese diets enhanced apparent calcium absorption. However, the added milk calcium increased calcium absorption for its respective cheese (Table 5). In terms of calcium digestibility (%A/I) a similar pattern emerged with caprine Ca-cheese > caprine cheese = bovine Ca-cheese > bovine cheese; again all cheese diets averaged 1.5 times that of the control diet. Note that fecal calcium is greatest for the control as well (Table 5) with caprine Ca-cheese diet being lowest.

For the groups of rats fed the caprine cheese diet and caprine Ca-cheese diet, the apparent magnesium absorption was significantly higher than those groups fed bovine cheese and bovine Ca-cheese diets; all cheese diets were again significantly higher than the control diet (Table 5). The cheese diets had an average absorption of $5.44\,mg\,d^{-1}$, which was 1.6 times that of the control; here added milk calcium did not enhance magnesium absorption of the respective cheese diets. Intake of caprine cheese and caprine Ca-cheese resulted in a significant increase ($P < 0.05$) in magnesium apparent digestibility (%A/I) when compared with bovine cheese, bovine Ca-cheese; the caprine cheese diets averaged 1.9 times that of the control diet and the bovine cheese diets averaged 1.4 times that of the control diet (Table 5), so that added milk calcium affected neither the magnesium absorption nor digestibility of the respective cheese diets. The pattern for magnesium is caprine Ca-cheese = caprine cheese > bovine Ca-cheese = bovine cheese > control.

3.2.3. Serum analysis

The concentrations of calcium in the blood serum (Table 6) increased significantly over control ($P < 0.05$) for the rats fed the caprine Ca-cheese diet and this level was greater than that of bovine Ca-cheese diet. When taken as a whole, the bovine and caprine cheese diets did not significantly increase serum calcium levels over control. Thus blood serum calcium concentrations were elevated only by the milk calcium fortification of the cheeses as shown in Table 3. This effect was not noted in the overall digestibility reported above (Table 5). Magnesium concentrations in the blood serum were significantly higher in the groups of rats fed the caprine cheese and caprine Ca-cheese diets in comparison to the groups of rats fed the bovine cheese diet or the control diet ($P < 0.05$). Here a species-specific response of the caprine cheese is noted.

Table 6
Concentrations of serum calcium (Ca) and magnesium (Mg) of rats fed the different diets for 28 days[a,b]

	Dietary group					
	Control	Bovine cheese	Bovine Ca-cheese	Caprine cheese	Caprine Ca-cheese	SEM
Ca ($mg\,dL^{-1}$)	7.52c	7.78c	8.21b	7.77c	8.56a	0.1
Mg ($mg\,dL^{-1}$)	0.80c	0.79c	0.82bc	0.89a	0.87ab	0.02

[a]See Table 1 and text for details of diet treatment.
[b]Mean values (±SEM; $n = 7$). Means not sharing common letters within each row are significantly different at $P < 0.05$.

Table 7
Femoral dry weight, ash, and mineral concentrations of rats fed the different diets for 28 days[a,b]

	Dietary group					
	Control	Bovine cheese	Bovine Ca-cheese	Caprine cheese	Caprine Ca-cheese	SEM
Dry weight (mg)	570d	601c	635b	633b	666a	9.9
Ash (mg)	287d	303c	318b	319b	333a	3.2
Ca ($mg\,femur^{-1}$)	87.3c	92.0bc	97.0b	98.9ab	105a	2.4
Mg ($mg\,femur^{-1}$)	4.67b	4.74b	4.70b	4.99a	5.13a	0.06
P ($mg\,femur^{-1}$)	48.5[b]	48.1[b]	48.7[b]	48.4[b]	48.2[b]	0.3

[a]See Table 1 and text for details of diet treatment.
[b]Mean values (±SEM; $n = 7$). Means not sharing common letters within each row are significantly different ($P < 0.05$).

3.2.4. Femoral mineral analyses

There were significant differences ($P < 0.05$) in the dry weight, as well as ash contents of the femoral bones among the groups of rats fed the experimental diets than in the group of rats fed the control diet (Table 7). For these two measurements, the pattern that emerged was caprine Ca-cheese > caprine cheese = bovine Ca-cheese > bovine cheese > control. It is interesting to note that this same pattern was observed for both calcium absorption and calcium digestibility (%A/I). In terms of actual calcium content, the group fed the caprine Ca-cheese diet had highest values which were not statistically distinct from the caprine cheese diet; here the bovine Ca-cheese diet was similar to the caprine cheese diet but only slightly different from the bovine cheese diet, which in turn was equal to the control. However, taken as a group, the caprine Ca-cheese, the caprine cheese and the bovine Ca-cheese are all significantly different from the control diet. The magnesium contents of the femoral bones of the groups of rats fed caprine cheese and caprine Ca-cheese diets were higher ($P < 0.05$) than all others, and the bovine cheese and bovine Ca-cheese diets were not different from those of the control diet (Table 7). Here a species-specific response of the caprine cheese diet similar to that of the serum magnesium level is noted. The femoral phosphorus concentration in the five experimental groups was not statistically significant (Table 7).

3.2.5. Bone mineral content (BMC) and bone mineral density (BMD)

The BMC of the femoral bones in the groups of rats fed the bovine cheese, caprine cheese, bovine Ca-cheese, and caprine Ca-cheese diets were higher ($P < 0.05$) than in the group of rats fed the control diet (Table 8). However, BMC was higher ($P < 0.05$) in the groups of rats fed the caprine Ca-cheese diets than those fed caprine cheese; in turn caprine cheese diets were > bovine Ca-cheese diets which were > bovine cheese diets. Similarly, the BMD in the groups of rats fed the bovine cheese, caprine cheese, bovine Ca-cheese, and caprine Ca-cheese diets were significantly higher ($P < 0.05$) than in the group of rats fed the control diet. Among the experimental diets, however, the caprine cheese and caprine Ca-cheese diet groups had higher BMD values ($P < 0.05$) than those of the bovine cheese and bovine Ca-cheese diet groups. The BMC and BMD values were significantly ($P < 0.05$) improved in both Ca-cheese diet groups when compared with the unfortified cheese diet groups for each species, respectively, which in turn was greater than the control diet. These patterns in general follow the responses seen for dry weight and ash contents, as well as calcium absorption and digestibility so the absorption patterns and deposition patterns follow the same overall order.

3.2.6. Breaking force

The breaking force of the excised femur bones in the groups of rats fed the caprine Ca-cheese diets were highest followed by the caprine cheese, bovine Ca-cheese, and the bovine cheese diets. All the cheese diets were significantly higher ($P < 0.05$) than in the group of rats fed the control diet (Table 8). In addition, the breaking force in the groups of rats fed the caprine cheese and caprine Ca-cheese diets were significantly higher ($P < 0.05$) than in the groups of

Table 8
Femoral bone mineral content (BMC), bone mineral density (BMD) and bone breaking force of rats fed the different diets for 28 days[a,b]

	Dietary group					
	Control	Bovine cheese	Bovine Ca-cheese	Caprine cheese	Caprine Ca-cheese	SEM
BMC (g)	189e	200d	211c	222b	234a	2.0
BMD ($g\,cm^{-2}$)	129e	137d	151c	160b	169a	2.1
Breaking force (kg)	8321e	8761d	9203c	9688b	10,191a	17.6

[a]See Table 1 and text for details of diet treatment.
[b]Mean values (±SEM; $n = 7$). Means not sharing common letters within each row are significantly different ($P < 0.05$).

rats fed the bovine cheese and bovine Ca-cheese diets. The breaking force values were also significantly ($P < 0.05$) improved in both Ca-Cheese diet groups when compared with the unfortified cheese diet groups for each species, respectively. This pattern follows exactly those of the BMC and BMD measurements and is in general similar to the calcium absorption and digestibility patterns. Again this emphasizes the concept that the breaking patterns, the deposition patterns and the absorption patterns follow the same overall order with respect to dietary treatment.

4. Discussion

Milk and dairy products represent the chief source of calcium in the typical American diet. Cheese is an excellent source of calcium because it contains absorbable calcium (milk calcium). In addition, the bioavailability of calcium from cheese is high because part of this calcium is complexed with the phosphoproteins in casein micelles (Bennet et al., 2000; Meisel et al., 2003). While there is some debate as to whether or not cheese is a better source of calcium than milk, as studies using isotope methods have questioned this effect (Buchowski & Miller, 1990; Recker, Bammi, Barger-Lux, & Heaney, 1988; Smith, Kolars, Savaiano, & Levitt, 1985), its innate ability to provide assimilable calcium has been supported (Kato, Takada et al., 2002).

Caprine cheeses made from milks with genetically variable casein contents have been shown to exhibit dramatic differences in their textural properties (Ambrosoli, Di Stasio, & Mazzocco, 1988). Moreover, it has also been shown that genetically variable caprine caseins also differ from those of bovine milk in terms of their interactions with calcium, and their resultant solubilities and colloidal stabilities (Mora-Gutierrez, Farrell, & Kumosinski, 1993, 1996). It was therefore of interest to determine if these in vitro effects would carryover to the cheese matrix and the digestive process. In this study, a semi-hard cheese (Monterey Jack type) was manufactured from both bovine and caprine milks with known casein compositions. To further test the potential calcium caseinate interactions, both milks were fortified with milk calcium derived from bovine milk, thus four cheese types were tested for bioavailability: caprine cheese, caprine Ca-cheese, bovine cheese, and bovine Ca-cheese. Note that the Ca-fortified cheeses are by their nature higher in calcium and magnesium than the non-fortified cheeses (Table 3), but the total minerals ingested were identical (Table 5) for all diets.

Proteolysis is recognized as one of the most important events during cheese ripening. Enzymatic hydrolysis of the different caseins by the action of plasmin, calf chymosin, and bacterial proteases produces a large number of peptides (Fox & McSweeney, 1996). Because proteolysis in cheese can yield differing peptide patterns with time we examined the time-dependent peptide patterns of the cheeses prepared in this study. Table 2 showed significant ($P < 0.05$) breakdown of the casein peptides in bovine and caprine Monterey Jack cheeses between the ripening periods of zero and 14 days. At 56 days of ripening, casein peptides tended to degrade even more rapidly ($P < 0.05$). Here we conducted the animal feeding study with bovine and caprine Monterey Jack cheeses ripened for 14 days. The study was performed within a time frame of 28 days so that the bovine and caprine cheeses had a total ripening period of 42 days at the end of the feeding study. Previous studies have shown that storage of Monterey Jack cheeses at 4 °C, slows proteolysis (Park & Jin, 1998). The fact that the casein peptides from bovine and caprine cheeses are relatively stable during this time frame (Table 2) allowed us to test the effects of variation of α_s-casein peptides (α_{s2}- and α_{s1}-casein) and β-casein peptides in bovine and caprine cheeses on the bioavailability of milk calcium. So the diets prepared as described in Materials and methods were composed of equal amounts of casein in similar states of proteolysis, but differed by increased bovine milk calcium phosphate in the Ca-fortified cheeses.

It is notable that all five of the experimental groups showed the same overall growth during the study regardless of calcium treatment (Table 4) and their daily food intakes were similar (Table 4). Differences among the groups were seen for the apparent absorption of calcium and magnesium (Table 5). In summary, the average calcium absorption for all cheese diets ($38.1\,mg\,d^{-1}$) was 1.5 times that of the control diet (Table 5), and calcium digestibility (%A/I) showed a similar pattern with caprine Ca-cheese > caprine cheese = bovine Ca-cheese > bovine cheese > control; again all cheese diets averaged 1.5 times that of the control diet. Thus cheese diets enhanced apparent calcium absorption, but the added bovine milk

calcium increased calcium absorption for its respective cheese (Table 5).

In contrast to the calcium data, magnesium absorption appeared to have a species-specific effect in that the two caprine cheeses were equivalent, but significantly different than the two equivalent bovine cheeses with the control lowest; in this case fortification with milk calcium did not alter the absorption of magnesium. It has been shown that the lipids present in the caprine cheese fat (rich in medium-chain fatty acids), favor the absorption and deposit of magnesium in the femoral bones of laboratory animals (López-Aliaga et al., 2003). Medium-chain fatty acids have been shown to be more easily absorbed into the intestinal lumen compared with long-chain fatty acids (Caspary, 1992). The fat in caprine milk is rich in medium-chain fatty acids ($C_{6:0}$ caproic, $C_{8:0}$ caprylic and $C_{10:0}$ capric comprise 15% versus 5% for bovine milk; Farrell, 1999); these fatty acids, which are absorbed in the proximal intestine, do not require bile salts to be absorbed (Vanderhoof, Grandjean, Kaufman, Burkley, & Antonson, 1984). Our data (Table 5) are in line with observations of Tantibhedhyangkul and Hashim (1978) who reported that magnesium soaps formed with medium-chain fatty acids are very soluble, and therefore, would favor magnesium absorption in premature infants. Furthermore, these results coincide with the widespread idea that medium-chain fatty acids favor magnesium absorption and utilization in rats with intestinal resection (Lisbona et al., 1994; López-Aliaga et al., 2003). The animals in our study that were fed the caprine cheese and caprine Ca-cheese diets showed differences in comparison with the groups of rats fed the bovine cheese and bovine Ca-cheese diets which could be due to the effect of the medium-chain fatty acids. It is interesting to note (Table 6) that the two caprine cheeses with or without fortification had increased serum magnesium levels, perhaps related to these same causes.

In contrast to magnesium, the fortification of the cheeses with bovine milk calcium led to increased serum calcium levels (Table 6), while the cheeses themselves were not different from the control in this respect. Milk calcium prepared by either the neutralization and precipitation method (hydroxypatite type) or by the ultrafiltration method (novel type) (Kato, Toba, et al., 2002) exhibits high calcium bioavailability in rats. At this point it may be asked whether or not the increased apparent absorption by the groups fed the cheese diets resulted in increased deposition as the cheeses alone appear to increase the apparent digestibility, but not the serum calcium level.

Significant changes ($P<0.05$) were noted in BMC and BMD in femurs of the rats fed the caprine Ca-cheese diet compared with those femurs of rats fed the bovine Ca-cheese diet, and the group of rats fed the caprine cheese diet had significantly higher ($P<0.05$) femoral BMC and BMD than the group of rats fed the bovine cheese diet (Table 8). All cheese diets were significantly greater than the control diet in BMC and BMD. The data also show that the caprine cheese and caprine Ca-cheese diets positively influence the biomechanical properties of the femoral bone in rats (Table 8). Thus, the femoral breaking force was significantly higher ($P<0.05$) in the groups of rats fed the caprine cheese and caprine Ca-cheese diets than in the groups of rats fed the bovine cheese and bovine Ca-cheese diets; the bovine diets were in turn also greater than the control diet in this respect. Thus lack of change in serum calcium levels for the cheese only samples may mean that they do not alter blood calcium homeostasis, but the absorption patterns seen for calcium (Table 5) where caprine Ca-cheese > caprine cheese = bovine Ca-cheese > bovine cheese > control is accentuated in the femoral breaking, BMC and BMD data of Table 8, where caprine Ca-cheese > caprine cheese > bovine Ca-cheese > bovine cheese > control.

The absorption, serum and deposition levels for magnesium argue for a species-specific effect as noted above. Boskey et al. (1992) demonstrated that feeding a magnesium-deficient diet ($20\,mg\,kg^{-1}\,diet^{-1}$) for 17 days resulted in a decrease in magnesium content of mataphyseal bone and a decrease in the maximum three-point bend strength of the femur in rats. A recent study by Shiga et al. (2001) demonstrated that massive large bowel resection (cecocolonectomy), which is an excision of the large bowel either partially, massively or totally, influences magnesium kinetics and decreases bone strength through reduction of the magnesium content of the femur in rats. The present results clearly suggest that the caprine cheese and caprine Ca-cheese diets strengthen the femur bones in rats by enhancing the amount of calcium and magnesium retained in bone femur (Table 7), and that this results from increased calcium and magnesium absorption which may depend in part on the high content of medium-chain fatty acids found in the fat fraction of caprine cheese (magnesium absorption). However, in all cases the casein containing diets were always greater than the control diets in the areas of absorption and deposition and breaking force. Since significant differences among the calcium levels occur between the bovine and caprine cheeses with and without calcium fortification these may be related to the innate differences in casein composition between the two species.

Components of milk, such as caseins, can alter mineral bioavailability. Caseins are phosphorylated at clusters of phosphoserine residues, which contain hydroxyl groups, in relatively close proximity in the amino acid chain (Farrell et al., 2004). While the bovine and caprine caseins used in this study have significantly different casein ratios, overall their phosphoserine contents are almost identical (26.4 and $27.1\,mmol\,100\,g^{-1}$, respectively) so one must look to the settings in which the phosphoserine residues occur.

Cheese is a fermented milk product so calf rennin in combination with bacterial proteases causes proteolysis in the cheese matrix. Further degradation of the caseins by digestive enzymes increases the likelihood of peptide liberation (Mahé, Marteau, Huneau, Thuillier, & Tomé, 1994). In vivo production of CPP by trypsin and other proteolytic enzymes in the gut may promote calcium

uptake by intestinal cells by preventing precipitation of insoluble salts (Bennet et al., 2000; Meisel et al., 2003). In fact CPP in vitro bind to and solubilize precipitating calcium phosphate nanoclusters (Holt, Timmons, Errington, & Leaver, 1998). Thus by increasing solubility the CPP would favor the absorption of calcium (Meisel, 1997; Meisel & Frister, 1989). The differences seen in this study could relate to the types of CPP liberated. In the caprine milk used in this study, 90% of the serinephosphates come from α_{s2}- and β-caseins, whereas in the bovine milk 78% come from α_{s1}- and β-caseins. Thus the CPP patterns from the two milks would be dramatically different and could account for the differences in absorption and distribution of calcium seen between the two cheeses with and without fortification. An additional factor could be the nature of the cheese matrix itself in that the caprine milk used in this study contains a high ratio of α_{s2}- to α_{s1}-casein, and caseins from such milks display a high internal hydration both at pH 4.8 and after exposure to rennet (Mora-Gutierrez & Farrell, 2001; Mora-Gutierrez et al., 1996). Increased internal water could be a factor in proteolysis and peptide diffusion.

The present work confirms the previous conclusions about the positive role of calcium fortification of bovine cheese with milk calcium on the mineral status of femoral bone in rats by Kato, Takada et al. (2002). Our findings also suggest that the efficiency of CPP produced in vivo after digestion of calcium-fortified cheese on the utilization of absorbed calcium for bone mineralization seems to depend on CPP type (bovine vs. caprine). Thus, CPP produced in vivo by digestion of calcium-fortified caprine cheese could result in a better utilization of absorbed calcium than CPP produced in vivo by digestion of calcium-fortified bovine cheese. It is generally believed that the clusters of phosphoserine residues are the determining factor for mineral binding, in particular calcium binding. Accordingly based on phosphorylation, the mineral binding activity of caseins follows the order: α_{s2}-casein $>$ α_{s1}-casein $>$ β-casein $>$ κ-casein (Kitts, 1994). In addition, it has been suggested that coordinate complexes may be formed between α_s- and β-casein molecules with calcium and other divalent cations, which lead to increased solubility (Farrell, 1999; Mora-Gutierrez et al., 1993). CPP released from casein hydrolysis in vivo have the ability to bind and keep soluble cations (Brulé & Fauquant, 1982); they improve calcium and zinc absorption (Hansen, Sandström, & Lönnerdal, 1996). The observation of differences in apparent calcium absorption (Table 5), which is strongly correlated with calcium solubility in the gut, between the bovine and caprine cheese diets at phosphorus intakes of 80.02 mg d^{-1} (bovine cheese diet), 79.25 mg d^{-1} (bovine Ca-cheese diet), 76.37 mg d^{-1} (caprine cheese diet), and 76.73 mg d^{-1} (caprine Ca-cheese diet) clearly suggest that chelation by phosphoseryl groups of their phosphoproteins was not equal. These results were expected because it has been reported that α_{s2}-casein has a high affinity for calcium (Kitts, 1994). In short, our findings suggest that in rats, calcium bound to caprine CPP characterized by a high content of α_{s2}-casein peptides displayed better absorption (Table 5) and better bone femoral uptake (Table 7) than bovine CPP; in turn the bovine CPP are superior to the non-casein control. The findings that calcium absorption was enhanced in rats after intake of caprine cheese unfortified or fortified with milk calcium are strong indication of the high bioavailability of α_{s2}-casein peptides-bound calcium. In addition, the high calcium content in the femoral bones of rats fed the experimental cheese diets is a good indicator of what occurs in the absorption and retention of calcium; it is noteworthy that the deposit was significantly greater when the rats were fed the Ca-cheese diets particularly the caprine Ca-cheese diet (Table 7).

The apparent increase in calcium and magnesium digestibility (Table 5) in rats consuming caprine cheese unfortified or fortified with milk calcium seems to alter the corporal calcium and magnesium content of the animals, as femoral bones presented higher quantities and concentrations of calcium and magnesium than bovine cheese unfortified or fortified with milk calcium (Table 7). These beneficial results may partly be explained by the greater digestibility of casein micelles from caprine milk (López-Aliaga et al., 2003). Thus, these animals indicated an increased calcium absorption capacity from caprine milk (and cheese) because of the higher quality of protein (or peptides) resulting from its animal origin (Pérez-Llamas et al., 2001). Moreover, the trend toward an increase in femoral bone magnesium concentration in the groups of rats fed the caprine cheese and caprine Ca-cheese diets may have been caused by CPP itself, which favors the deposit of divalent cations (López-Aliaga et al., 2003). Caprine milks are also characterized by a high content of β-casein (see Materials Section 2.3), and a high β-casein/κ-casein ratio has been linked to a better digestibility of colostrum casein micelles from human milk, which is rich in β-casein, by the neonate during the first days of life (Cuilliere, Tregoat, Bene, Faure, & Montagne, 1999).

Although the results presented here were obtained with bovine and caprine Monterey Jack cheese samples, it is believed that the findings generated in this study may relate to other cheeses coagulated with calf chymosin (rennin) alone or in combination with acid from bacterial starters.

5. Conclusions

Our data suggest that caprine Monterey Jack cheese characterized by a high content of α_{s2}- and β-casein peptides, unfortified or fortified with milk calcium, may be more protective against the bone fragility than bovine Monterey Jack cheese most likely by enhancing calcium and magnesium absorption in the gut. However, the bovine diets were also more effective than the non-casein control diet in calcium uptake, deposition and femoral breaking force. The bovine cheese diets lacked the ability to enhance magnesium deposition, so there may be a positive

protective role of medium-chain fatty acids, which are found in high amounts in the fat fraction of caprine dairy products, on the mechanical properties of bone by enhancing magnesium absorption. Magnesium is closely associated with bone calcium and phosphorus metabolism (Illich & Kerstetter, 2000). Caprine cheese unfortified or fortified with milk calcium may be of help for those people with low calcium absorption (Heaney, Saito, & Orimo, 1994); however, clinical studies in human subjects are required to confirm the applicability of these results.

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